969 Thrombosis, Atherosclerosis, and Vascular Biology: Atherogenesis, Platelet and Cell Growth

Tuesday, March 26, 1996, Noon–2:00 p.m.
Orange County Convention Center, Hall E
Presentation Hour: 1:00 p.m.–2:00 p.m.

969-109 Inhibition of Atherosclerosis and Xanthoma Formation by L-Arginine in Cholesterol-Fed LDL Receptor Knockout Mice

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To investigate antiatherosclerotic actions of nitric oxide (NO), experiments were performed in 4 groups of mice (n = 5/group) lacking functional LDL receptor genes (LDLR-ko). Group 1 was fed a regular chow diet. Groups 2 to 4 were fed a 1.25% high cholesterol Paigen diet; in addition, group 3 received supplemental L-arginine (arg) and group 4 received arg plus L-nitro arginine (LNA), an inhibitor of NO synthase. Animals were sacrificed at 6 months; hearts and aortas were removed and stained with oil-Red O for planimetry.

Cholesterol was markedly increased in all animals on the high cholesterol diet compared to controls. Lipid profiles were similar in groups 2–4. Xanthomas appeared in mice fed the high cholesterol diet and in those fed the high cholesterol diet plus arg and LNA but not in the controls or in mice given arg. The mean atherosclerotic area was reduced significantly in the cholesterol-fed LDLR-ko mice given supplemental arg in comparison to those receiving the high cholesterol diet alone (Table).

Group	Chow	High Chol	arg	arg + LNA
Lesion area μ^2	0	797,053	419,651	1,161,833

$P < 0.005$ Student t-test

The data indicate that arg supplementation inhibits atherosclerosis and xanthoma formation in LDLR-ko mice on a high cholesterol diet. The fact that LNA abrogated these effects suggests that the actions of arg are mediated by NO synthase. The data raise the possibility that arg may be beneficial in patients with familial hypercholesterolemia.

969-110 First Experience With Chronic Platelet GPIIb/IIIa Receptor Blockade: A Pilot Study of Xemlofiban an Orally Active Antagonist in Unstable Angina Patients Eligible for PTCA

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Although GPIIb/IIIa blockage has considerable promise in ischemic heart disease, there has been no experience with oral sustained inhibition to date. We performed a placebo controlled pilot study to evaluate the safety and pharmacodynamic response of Xemlofiban (X) administered orally (25 mg tid) for one month in patients with unstable angina undergoing PTCA. Of 23 pts entered into the study, 15 were randomized to receive X and 8 to placebo (Pl). Of 15 pts on X, 4 were withdrawn during the initial hospitalization: groin hematoma (n:2), stent implantation (n:1) and severe GI bleeding (n:1). Two subjects withdrew later because of rectal bleeding. Of the 9 pts who completed the study, 8 reported minor bleeding events. Pharmacodynamic studies showed rapid onset, profound and sustained inhibition of platelet aggregation compared to aspirin.

Platelet Aggregation (%)

	Pre		2 Hrs post		2 Weeks		4 Weeks	
	PL	X	PL	X	PL	X	PL	X
ADP (20 μM)	63±28	68±21	68±32	26±33	54±32	6±6	56±34	18±29
Collagen (4 μg/ml)	51±31	47±28	59±33	20±26	38±31	6±2	54±37	18±22

Conclusion: Xemlofiban given orally results in potent inhibition of platelet aggregation for a period up to one month. Minor bleeding events are common. Severe bleeding in 1 pts was associated with prolonged elimination of the drug. *This aspect deserves further study.*

969-111 Transforming Growth Factor-Beta 1 Induces the Expression of Vitronectin in Smooth Muscle Cells

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Vitronectin (VN), an extracellular matrix adhesive protein, regulates the coagulation, complement, immune and fibrinolytic systems. VN can potentially alter the growth of the neointima by its ability to act as a haptotactic factor for smooth muscle cells (SMC), its ability to prolong the activity of thrombin, and its stabilization of plasminogen activator inhibitor-1. We have shown that vitronectin is present in atherosclerotic and restenotic coronary arteries by performing immunohistochemistry of coronary artery sections and atherectomy samples. Using RT-PCR, we have further shown that vitronectin is expressed by cells within the vessel wall, rather than merely being deposited from plasma. Based on its distribution, we hypothesized that VN was expressed by SMC. SMC were cultured until confluent, then serum-starved them for 3 days, prior to incubation with vehicle (unstimulated), TGF-β1 (10 ng/ml), α-thrombin (20 nM) or basic FGF (10 ng/ml) for 18 hours. Total RNA was harvested, treated with DNAse, then used for cDNA synthesis with AMV reverse transcriptase and oligo dT priming. Specific primers for VN were used for PCR. Using the RT-PCR method, there was no VN expression in unstimulated or bGGF-stimulated cells and only a faintly detectable band with α-thrombin stimulation. In contrast, TGF-β1 clearly produced a marked induction of vitronectin expression in the SMC. In conclusion, the expression of VN is induced by TGF-β1 in smooth muscle cells, probably explaining its origin in atherosclerotic and restenotic coronary arteries. Vitronectin expressed by SMC in the vessel wall may contribute to neointimal growth.

969-112 Reduction of Cyclic Flow Variations by Endothelin B Receptor Stimulation Is Mediated by Nitric Oxide in Rat Mesenteric Artery Model

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Background: Cyclic flow variations(CFVs) represent repetitive cycles of platelet adherence-aggregation, followed by dislodgement of platelet thrombi and restoration of blood flow at the site of vascular injury. We have shown that endothelin A receptor blockage reduces and endothelin B receptor (ETB) blockage promotes CFVs in rat mesenteric artery model. Since ETB stimulation leads to nitric oxide (NO) release from vascular endothelial cells, we hypothesized that effect of ET on CFVs is mediated by NO. *Method:* Male Wistar rats (250–274 g) were anesthetized with pentobarbital. The side branch of mesenteric artery (200–350 μm) was cannulated with a polyethylene catheter. A short segment of artery was mechanically injured. CFVs and % luminal stenosis were recorded using intravital microscopy. After 20 minutes of saline infusion (1 uL/min, base line phase), saline (negative control, n = 8) or Sarafotoxin S6C (S6C, 10 ng/mL, ETB *agonist*, N = 12) was infused for 20 min (1 uL/min, interventional phase). Six rats with S6C also had L-NAME (*Nitric oxide synthesis inhibitor*, 10^{-5} M) superfused over the vessel segment. This was followed by a second saline infusion for 20 min (recovery phase). The % changes of CFVs were calculated. *Result:* While saline infusion did not change CFVs (0.8 ± 4.6% SE), S6C infusion reduced the CFVs by 26.5 ± 10% ($p < 0.05$ compared with saline control). Superfusion of L-NAME not only abolished the effect of S6C but tended to increase the CFVs (15.9 ± 6.6%, $p > 0.3$ *compared with saline control*). *Conclusion:* ETB stimulation reduces CFVs through nitric oxide synthesis since its effect is abolished by L-NAME, nitric oxide synthesis inhibitor.

969-113 Biphasic Mural Proteolysis in Atherogenesis

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Plasminogen activators (PAs, tissue type [t-PA] and urokinase type [u-PA]) and their primary inhibitor (type 1 [PAI-1]) may modulate progression of atherogenesis by facilitating accumulation and degradation of extracellular matrix. In this study, we determined the tissue content of PAs and PAI-1 in paired samples of normal and atherosclerotic arteries from the same patient. The tissue obtained during surgery and maintained in organ culture demonstrated viability by continued protein and DNA synthesis. After culture, protein was extracted and analyzed by ELISA for content of t-PA, u-PA, and PAI-1. In the first pair, a fatty streak was compared with adjacent 'normal' tissue from an aorta. PAI-1 content was increased by 15.6 ng/mg tissue protein (prot) and PA content was decreased by 0.6 ng/prot in the fatty streak. The second pair comprised a popliteal artery segment with 50% stenosis and a 'normal' branch of the same vessel. PAI-1 content was decreased by 11.9 ng/prot and PA content was increased by 0.44 ng/prot. The third pair comprised a superficial femoral artery segment with a > 90% stenosis and a 'normal' collateral. PAI-1 content was increased by 8.6 ng/prot and PA content was increased by 1.13 ng/prot consistent with increase PA content seen in severely diseased arteries (> 80% stenosis) compared with 'normal' arteries (diseased PAs = 4.9 ± 1.7, n = 10; 'normal' PAs = 0.9 ± 0.2 n = 8; ng/prot, $p < 0.02$). Thus, decreased mural proteolysis in early atherosclerosis may potentiate extracellular matrix accumulation and the subsequent increased mural proteolysis in moderate and severe atherosclerosis may be a marker of or potentiate migration of macrophages and smooth muscle cells.